

Voltage-gated ion channels play crucial roles in the generation and propagation of electrical signals within the nervous system. Peptide toxins from tarantula venom interact with voltage-sensing domains in Kv channels and have provided useful tools for exploring their structures and gating mechanisms. However, relatively little is known about the specific molecular interactions between these toxins and voltage sensors. In the present study we set out to define the surface of GxTx-1E that interacts with the voltage sensors in the Kv2.1 channel. GxTx-1E is a particularly attractive candidate for further study because the structure of this amphipathic toxin has been solved, and it binds with high affinity to the voltage-sensor paddle motif in the Kv2.1 channel to inhibit opening, providing the opportunity to undertake mutant cycle analysis between toxin and channel. Towards this goal we mutated 28 of the 36 non-Cys residues in GxTx-1E to Ala using solid-phase chemical synthesis, folded the mutants *in vitro* and studied their interactions with the Kv2.1 channel using voltage-clamp electrophysiological recording techniques. Of all the mutations studied thus far, none of the polar residue mutants produced changes in toxin affinity greater than 5-fold, whereas five hydrophobic residue mutants produced > 10-fold changes in toxin affinity. In the structure of the toxin, all of these hydrophobic residues are positioned to form a contiguous surface. We are currently undertaking mutant cycle analysis between these mutants and those within the paddle motif to define the protein-protein interface between toxin and channel.

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Opening the Shaker Kv Channel with Hanatoxin

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Voltage-activated ion channels open and close in response to changes in membrane voltage, a property that is fundamental to the roles of these channels in electrical signaling. Protein toxins from venomous organisms commonly target the S1-S4 voltage-sensing domains in these channels and modify their gating properties. Studies on the interaction of hanatoxin with the Kv2.1 channel show that this tarantula toxin interacts with the S1-S4 domain and inhibits opening by stabilizing a closed state. Here we investigated the interaction of hanatoxin with the Shaker Kv channel, a voltage-activated channel that has been extensively studied with biophysical approaches. In contrast to what is observed in the Kv2.1 channel, we find that hanatoxin shifts the conductance-voltage relation to negative voltages, making it easier to open the channel with membrane depolarization. Although these actions of the toxin are subtle in the wild-type channel, strengthening the toxin-channel interaction with mutations in the S3b helix of the S1-S4 domain enhance toxin affinity and cause large shifts in the conductance-voltage relationship. Using a range of previously characterized mutants of the Shaker Kv channel, we find that hanatoxin stabilizes an activated conformation of the voltage sensors, in addition to promoting opening through an effect on the final opening transition. Chimeras in which S3b-S4 paddle motifs are transferred between Kv2.1 and Shaker Kv channels, as well as experiments with the related tarantula toxin GxTx-1E, lead us to conclude that specific interactions between toxins and paddle motifs determine whether these toxins inhibit or promote channel opening.

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The Energetic Landscape of the Putative Voltage-Driven Conformational Transition in Ci-VSP

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Two recent x-ray crystal structures of Ci-VSP have, for the first time, captured both the putative up and down states of a Voltage-Sensing Domain (VSD). This structural information provides a first chance to characterize the nature of the voltage-driven conformational transition using molecular dynamics simulations based on detailed atomic models. Here, using umbrella sampling potential of mean force (PMF) calculations, we explore the free energy landscape governing the transition of the S4 helix between these two states of the Ci-VSP voltage sensor. PMFs calculated with an applied transmembrane potential helps elucidate the voltage dependence and the gating charge of the Ci-VSP sensor in a lipid bilayer membrane.

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Structural Basis of Lipid-Driven Conformational Transitions in the KvAP Voltage Sensing Domain

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Voltage-gated ion channels respond to transmembrane electric fields through the motion of the positively charged S4 helix present in the voltage-sensing domain (VSD). In doing so, they are responsible for the electrical and chemical signaling that underlies cellular communication and signal transduction. VSDs respond to changes in the membrane potential by moving charged residues in the S4 helix through the focused electric field of the membrane. Despite structural data, the details of this conformational change remain unresolved. Previous studies (1, 2) demonstrated that non-phosphate lipids can dramatically right-shift the voltage dependence (G-V curve) of KvAP, trapping the VSD in a putative resting (Down) state while reconstitution in phospholipids leads to the stabilization of the activated (Up) state. We have evaluated the conformations of KvAP's isolated VSD through reconstitution in lipids with (PC:PG) or without (DOTAP) phosphate groups. The nature of the structural transition between these states was determined using site-directed spin labeling and EPR spectroscopy. Solvent accessibility and inter-helical distance determinations suggest that KvAP gates through a novel mechanism involving a ~3 Å upward tilt and simultaneous ~2 Å axial shift of S4. This motion leads to large accessibly changes in the intracellular water-filled crevice, which offers an alternative explanation of previous findings and supports a novel model of gating that combines structural rearrangements and local field refocusing.

1. D. Schmidt, Q. X. Jiang, R. MacKinnon, *Nature* **444**, 775 (Dec 7, 2006)

2. H. Zheng, W. Liu, L. Y. Anderson, Q. X. Jiang, *Nat Commun* **2**, 250 (2011)

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A Molecular Mechanics Model of a Closed Voltage-Gated Potassium Channel Generated from S4-S5 Linker LRET Measurements

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Voltage-gated potassium ion (Kv) channels regulate action potentials of the nervous system by responding to changes in transmembrane voltage, enabling K⁺ transport across the membrane to restore cells to their resting potential. While several crystal structures of Kv channels have been presented in the open conformation, the closed structure remains unsolved, leaving the Kv channel gating mechanism unclear. Using lanthanide-based resonance energy transfer (LRET) measurements of the S4-S5 linker, which connects the voltage sensor to the pore domain, we modeled the Kv 1.2/2.1 chimera in the open and closed conformations. Through fully atomistic molecular dynamics simulations of the models, we find that a small 4 Å displacement of the linker is sufficient to gate the channel. Additionally, we find a 9 Å vertical translation of S4, and a 37° change in tilt of S4 with respect to the S4-S5 linker between the open and closed states, in agreement with previously published studies. Here, we present the first model of the closed channel derived from measurements on the cytosolic side of the channel.

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Tracking a Voltage-Sensor Cycle with Models of Intermediate States from Metal-Ion Bridge Constraints

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Voltage-gated ion channels enable electric signaling by responding to changes in membrane potential. This is controlled by four voltage-sensor domains (VSDs) in which the fourth transmembrane segment (S4) contains several positively charged residues that move in response to voltage changes. An open conformation is available from the Kv1.2/2.1 X-ray structure, and several recent simulations based on experimental constraints have lead to an emerging consensus model of the resting state. We have extended this approach by systematically exploring residue contacts that should occur during the VSD gating, and tested these with electrophysiology. By using metal-ion bridges that are weaker than disulphides it is possible to keep the channel working and quantify shifts in voltage dependence. We report a total of 20 new interactions, which more than double the number of experimental constraints available for VSDs, and classify them into one open and three successively more closed intermediates. A subset of constraints was used to build models of each conformation with Rosetta, and after subsequent simulation (without constraints) the models fulfill all constraints in each state. Further, under some conditions it appears to be possible to drive Shaker into an even deeper fourth closed state for which we also provide a model. Molecular simulations show that these intermediate states indeed correspond to metastable conformations. Starting from the first closed state and driving the S4 helix upwards in a simulation results in stable conformations within 3 Å RMSD of the experimental open state structure. These results provide insight